Impact of Mild Noise Exposure on Cochlear Auditory Evoked Potentials

Undergraduate Research Thesis

Presented in partial fulfillment of the requirements for graduation with research distinction in Speech and Hearing Science in the undergraduate colleges of The Ohio State University

By

Kayla Fuzer

The Ohio State University

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Project Advisor: Eric Bielefeld, Ph.D., Department of Speech and Hearing Science

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Abstract

Noise that induces threshold shift, either permanent or temporary, has been shown to induce significant changes in the afferent pathway from the cochlear hair cells to the auditory nerve. This noise-induced de-afferentation of the cochlea can be detected and assessed using Electrocochleography (EcochG), a set of auditory evoked potentials that reflects activation of the cochlea by sound. Whether threshold shift is required to induce de-afferentation of the cochlea is unknown. The purpose of the current experiment was to examine the effect of a one-time mild noise exposure that does not induce threshold shift on the cochlear afferent pathway from the inner hair cells (IHC). In this study, a group of seven male and female Fischer 344 rats were tested. These animals were exposed to a one-time noise exposure (narrowband noise centered at 10 kHz with a bandwidth of 10 kHz presented at 70 dB SPL) that was not intense enough to cause either a temporary or permanent threshold shift. The animals were then tested with EcochG in order to monitor the cochlear microphonic (CM) and the compound action potential (CAP) components to detect any cochlear de-afferenation induced by noise. The testing was repeated once per month for a period of six months to determine any long term changes in the cochlear physiology as a result of the noise exposure. The results demonstrated that significant differences were detected in the CAP amplitudes after the noise exposure, but there were no changes in the CM-CAP ratio over the test period of six months. The stability of the CM-CAP ratio suggests that threshold shift is required for a noise to induce significant cochlear de-afferentation.

Introduction

Noise exposure is known to cause varying levels of hearing loss as well as a number of different cochlear pathologies. Ultimately, the different levels of noise exposure directly contribute to noise-induced hearing loss (NIHL) and its effects on specific individuals and contemporary society as a whole. NIHL is the result of damage to the sensitive structures within the cochlea, and more specifically the hair cells. Noise exposure can damage or kill both the inner hair cells (IHC) and outer hair cells (OHC) causing temporary threshold shifts as well as permanent threshold shifts (Henderson et. al., 2006). Loss of the OHC function exhibits a parallel relationship with the differing degrees of hearing loss. In contrast, the effects of noise on the IHC are much less well understood. IHC are responsible for delivering the bulk of incoming auditory information to the auditory nerve, where the information is then delivered to the brain. Each IHC has several synaptic connections with afferent auditory nerve dendrites. Those synapses show swelling and loss of connections under the IHC after noise exposure (Pujol et al., 1993; Puel et al., 1998). This de-afferentation of the IHC and the auditory nerve dendrites leads to an eventual decay of the spiral ganglion cell bodies from which those afferent dendrites project (Kujawa & Liberman, 2006; Lin et al., 2011). Interestingly, this can occur even after noise exposure that causes only a temporary threshold shift. Previously, those temporary threshold shifts were believed to be completely innocuous and even potentially beneficial to the ear. Loss of the spiral ganglion cells and loss/damage of their connections to the IHC can be detrimental to the processing of complex auditory signals. These effects can significantly inhibit one's speech processing abilities (Kujawa & Liberman, 2009).

Consequently, the testing and examination of spiral ganglion cell loss is extremely important in the prevention of impaired speech discrimination ability. There is currently no test

available to specifically address the spiral ganglion cells and their connections to the IHC. There exists a need for a procedure to analyze these specific areas of the cochlea. The use of an Electrocochleography (EcochG) laboratory test, will allow the comparison and evaluation of electrical potentials evoked from sound stimulation. The EcochG produces three potentials consisting of the cochlear microphonic (CM), summating potential (SP), and the compound action potential (CAP) (Hall, 2007). For the purposes of focusing on spiral ganglion cell degeneration, this study will focus on the comparison of the CM and CAP to test the spiral ganglion neurons. The CM reflects electrical potentials generated by the OHCs, and the CAP results from summed action potentials of the spiral ganglion cells and their excitation by the IHCs. In cases of noise-induced spiral ganglion cell loss, the CAP component of EcochG should decline while the CM component remains stable. Therefore, the CM-CAP should increase. In cases of OHC damage, both the CM and CAP amplitudes will decline, resulting in no change in the CM-CAP ratio.

As discussed above, IHC de-afferentation and spiral ganglion cell loss occurs even after noise exposures that do not induce permanent threshold shift (Kujawa & Liberman, 2009). This recent discovery has the potential to cause a complete re-examination of modern noise protection standards. What is currently unknown is whether noise that induces no detectable threshold shift (either temporary or permanent) has any effect on IHC de-afferentation. This study ultimately aims to test the relationship between the CM and the CAP overtime after one mild noise exposure. The working hypothesis for the experiment is that the CM and CAP amplitude input-output functions can be used to assess the long-term cochlear de-afferentation following any sound exposure, and the CM to CAP ratio should remain stable after a noise exposure that induces no threshold shift, because no cochlear de-afferentation would occur after such a noise

exposure. The expectation is that noise must induce at least temporary threshold shift in order for the long-term IHC de-afferentation and spiral ganglion loss to occur.

Materials and Methods

Subjects

The study tested seven 2-3 month old, male and female, Fischer 344 rats. The animals were housed together at The Ohio State University and closely monitored. The rats' cochlear reactions are similar to reactions seen in humans. However, it is important to note that Fischer 344 rats are known to hear a whole octave above normal human hearing. As a result, the audiometric testing and noise exposure were done at frequencies higher than normally would be used in humans. All procedures involving the care and use of the animals were approved by the university's Institutional Animal Care and Use Committee.

Procedure

The animals were tested using the CM and CAP components of EcochG. To record the EcochG electrical potentials, sub-dermal needle electrodes were used. The inverting electrode was placed directly behind the pinna of the test ear as close to the auditory bulla, or bony structure as possible. The non-inverting electrode was placed behind the pinna contralateral to the test ear. The ground electrode was placed on the animal's back. Test stimuli were toneburts of 5, 10, and 15 kHz frequencies. The stimuli were delivered from a speaker positioned 3 inches from the test ear of each animal. These acoustic stimuli were calibrated before each testing and Tucker Davis Technologies BioSig RZ programming was used for stimulus delivery and data collection. Each toneburst had a duration of 1ms with a 0.5 ms rise/fall time with no plateau. The signal level was decreased in 5 dB steps beginning at 100 dB SPL and decreasing to 5 dB SPL.

Once pre-tested, the rats were exposed to a mild noise exposure consisting of a narrowband noise centered at 10 kHz with a bandwidth of 10 kHz presented at a sound pressure level of 70dB SPL for 30 minutes. This exposure was completed once and was intended to not be

powerful enough to cause temporary or permanent threshold shift in the rats. Following the noise exposure, the animals were tested for CM and CAP amplitudes once per month for six months. Completing the test in this way allowed the rats to be monitored for CM and CAP to determine if there are any long-term changes in cochlear physiology as a result of the noise exposure.

Predicted Results

The rats were not expected to exhibit any temporary or permanent threshold shift as a result of the noise exposure. The CM response was not expected to change significantly at any point in the six-month period after the noise exposure. The CAP was also expected to remain stable over the six-month test interval. Since the CM and CAP amplitudes were both expected to remain stable, there were no significant changes in the CM/CAP ratio anticipated over the six-month observation interval. However, the fact that the CAP declines over time after a temporary threshold shift-induced noise did introduce the possibility that it could decline over time in the current study. If there were any effects of the noise exposure on cochlear de-afferentation, they would manifest as a stable CM and a decrease in the CAP, leading to an increase in the CM/CAP ratio.

Results

After the completion of the six month testing period, the CM and CAP amplitude functions were measured, and data were analyzed. A two-factor ANOVA with repeated measures was used to assess the differences between test times at each stimulus level. Each frequency was assessed separately. The CAP amplitude input-output functions and CM/CAP amplitude ratios were used as dependent variables. CAP amplitude input-output functions for 5, 10, and 15 kHz are displayed in Figures 1, 2, and 3 respectively. Significant effects ($p < 0.05$) of stimulus level were detected in all recordings. This was anticipated since the higher stimulus levels provoke higher CAP amplitudes than the lower levels. Since the effect was expected, no post hoc analyses were performed to assess the effect further. No significant differences were detected in test time for the 5 kHz stimulus, but significant main effects of test time were detected at 10 kHz and 15 kHz. Post-hoc analyses revealed that the pre-exposure amplitudes were significantly

higher than the post-exposure amplitudes at each of the test times (4, 8, 12, 16, 20, and 24 weeks post noise).

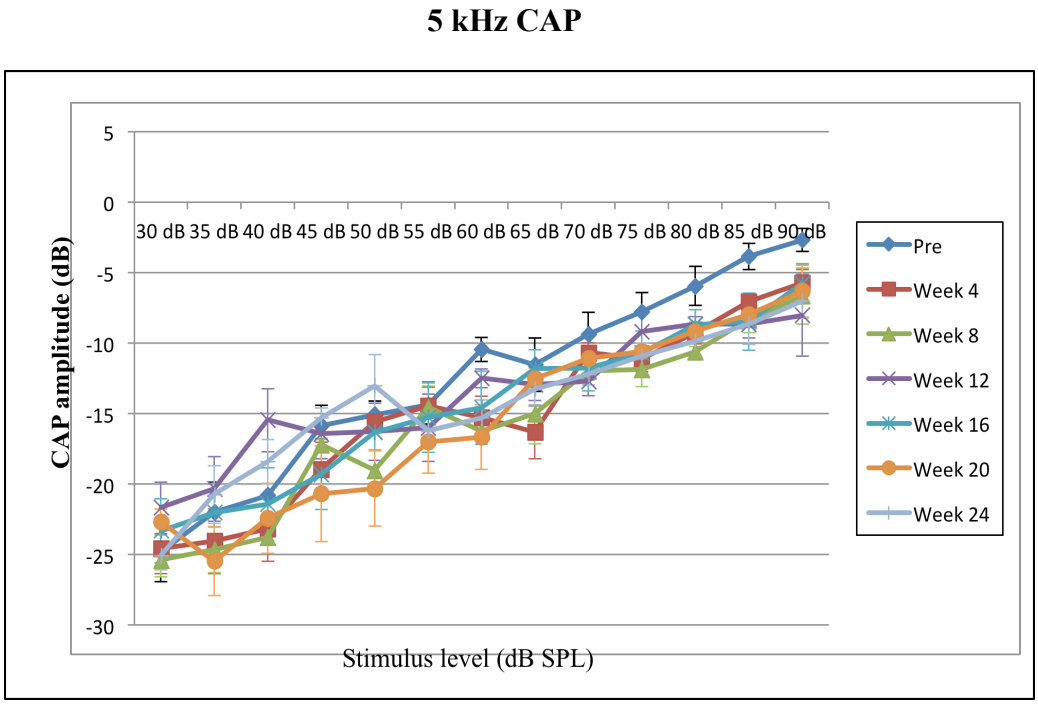

Figure 1: CAP amplitudes at 5 kHz across all test points. A statistical trend (p=0.064) toward a main effect of test week was detected, but no significant (p< 0.05) main effect or interaction involving test week was detected.

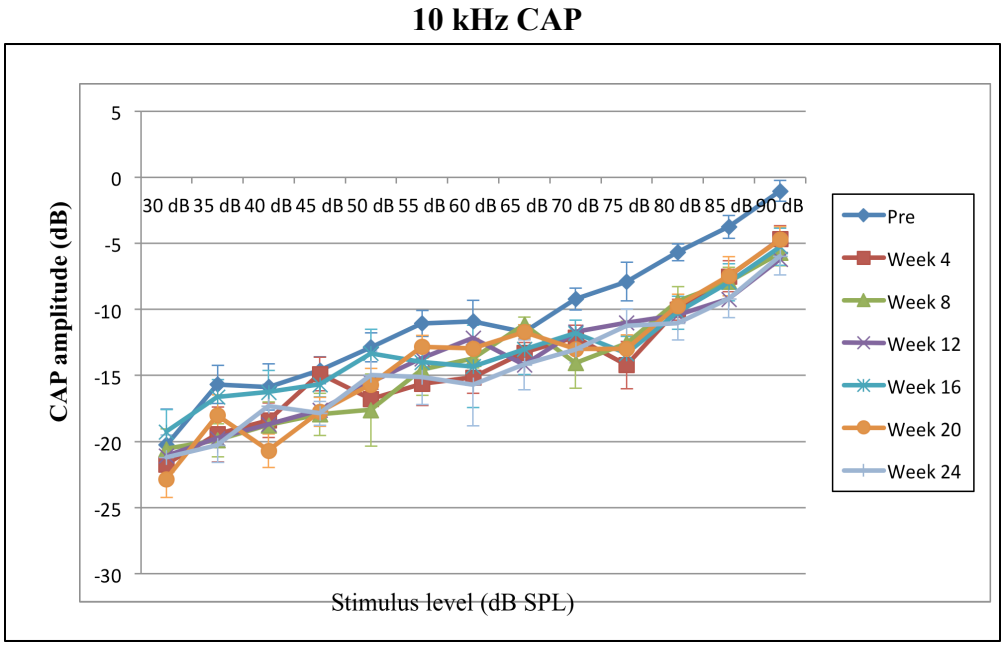

Figure 1: CAP amplitudes at 10 kHz across all test points. A significant main effect ($p<0.05$) of test week was detected, with the pre-exposure amplitudes being higher than all post-exposure amplitudes

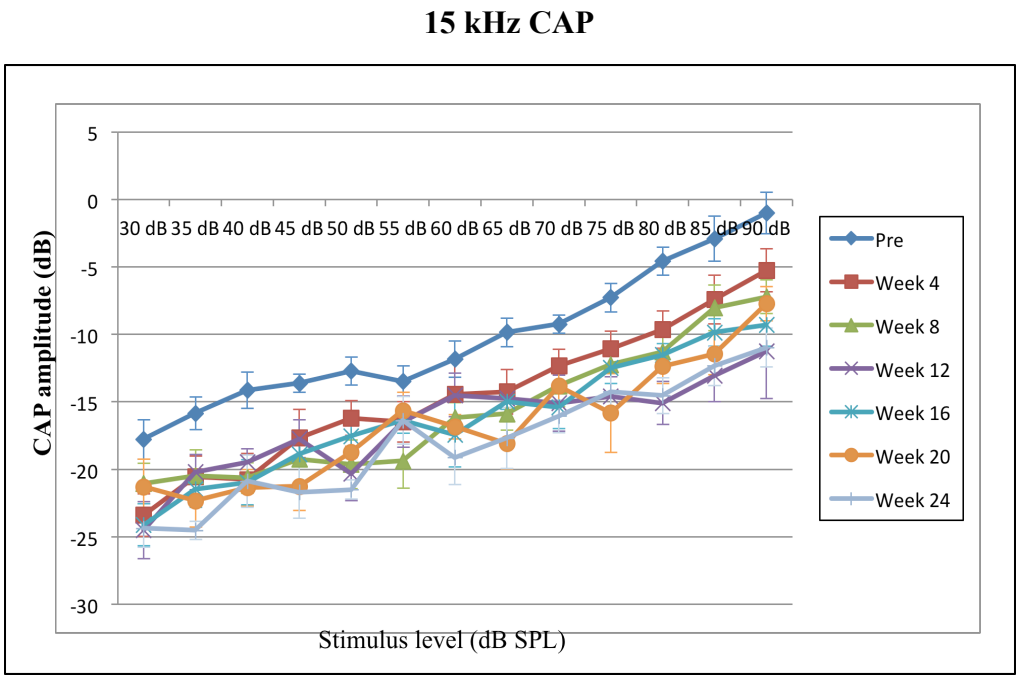

Figure 3: CAP amplitudes at 15 kHz across all test points. A significant main effect ($p<0.05$) of test week was detected, with the pre-exposure amplitudes being higher than all post-exposure amplitudes

The CM/CAP ratios were calculated for 70-90 dB SPL, as those were the stimulus levels in which the CM was robust enough to be consistently calculated. The CM/CAP ratio components exhibited no significant changes. A statistical trend (p=0.084) for an effect of test time at 15 kHz was detected, but it was not significant. The lack of significant changes with the CM/CAP ratio components implies that when the CAP amplitude was high/low the CM amplitude was high/low; therefore the CM/CAP ratio exhibited no change.

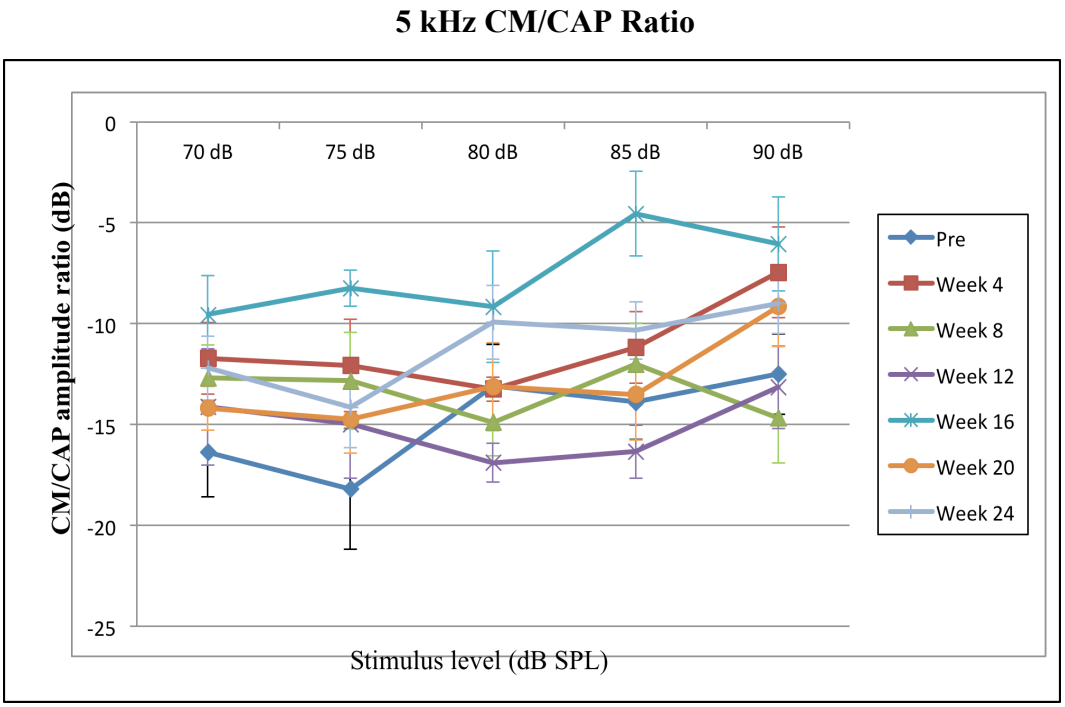

Figure 4: CM/CAP amplitude ratios at 5 kHz across all test points. No significant differences were detected in the data.

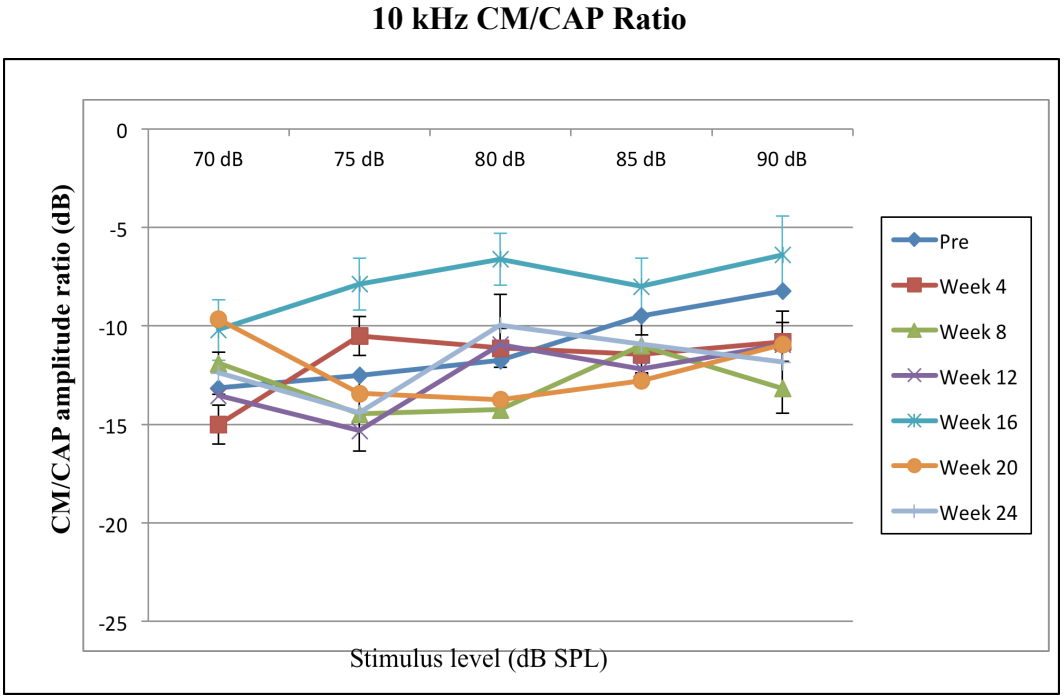

Figure 5: CM/CAP amplitude ratios at 10 kHz across all test points. No significant differences were detected in the data.

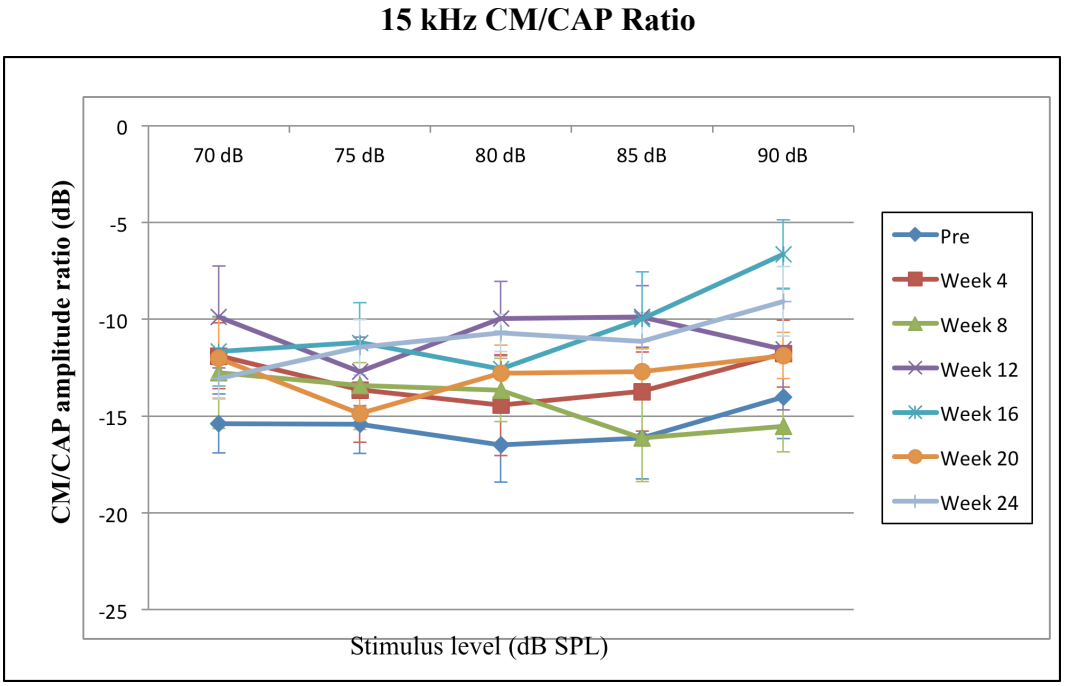

Figure 6: CM/CAP amplitude ratios at 15 kHz across all test points. No significant differences were detected in the data.

Discussion

The reduction in CAP amplitudes after the noise was not consistent with the Predicted Results, though the stable CM/CAP ratios from those recordings were consistent with the expectations. The depressed CAP amplitudes were attributable to two animals' data that had much higher CAP amplitudes in the pre-exposure test condition than any other animals or any other test points. Specifically, subject number 7 and 8 exhibited significant pre-exposure amplitudes that their data points had an influential effect on the entirety of the test data, demonstrating a significant decrease in the CAP amplitude functions on all future recordings. Further examination is required to determine if these data were the result of a testing error or if it was a unique attribute of the test animals.

The stability of the CM/CAP ratio over time indicates that when the CAP was higher in the pre-exposure test, that the CM was also higher. Thus, the CM/CAP was stable. This further indicates that the pre-exposure recordings of animals 7 and 8 were unique anomalies, rather than truly clinically significant results. In addition, pre-testing of animals 7 and 8 occurred on one testing session, and no other animals were tested during that same session. Ultimately, the CM/CAP may serve as a better measurement tool when testing one test subject over a long period of time, since it does not seem to be as heavily affected by the seemingly random data fluctuations as the CAP measurements tend to be.

The rats did not exhibit any temporary or permanent threshold shift as a result of the noise exposure. The noise was not intense enough to cause any significant cochlear de-afferentation. However, the results can be interpreted in a variety of ways in addition to the above interpretation that two outlier recordings affected the overall data. The significant main effect of test time at 10 kHz and 15 kHz can be attributable to a variety of other factors. One

possibility is that the rats had in fact developed an ear infection causing some level of a conductive hearing loss. The animal subjects are housed in an animal colony and there is a possibility that their health and their hearing levels were compromised. This conductive hearing loss could have had a significant effect on our post-exposure amplitudes causing a decrease in our CAP amplitudes. If this were the case, the CM would also have been affected, and the CM/CAP ratio would be stable, which it was in the current experiment.

Additionally, the significant decrease in post-exposure CAP amplitudes could in fact be attributed to the effect of noise. This is not believed to be the case due to the lack of significant changes in the CM/CAP ratio. With cochlear de-afferentation, an increase in the CM/CAP ratio would occur. The lack of significant changes in our CM/CAP ratio provides evidence that the decrease in post-exposure CAP amplitude functions in 10 kHz and 15 kHz is not attributable to noise-induced cochlear de-afferentation. But the possibility of direct damage to the OHCs could have occurred from the noise. Effects on the OHCs would reduce both the CM and CAP amplitudes, leading to the pattern of results in the current study. Further examination of the rats' cochlea should be conducted to determine if the decrease in our CAP amplitudes was due to a testing error, a unique attribute of the test subjects, or a direct effect of the noise on the OHCs. Overall, it is not believed that the noise was intense enough to affect cochlear de-afferentation or the OHCs. Based on the findings, we support the hypothesis that noise needs to be intense enough to induce either a permanent or temporary threshold shift in order to affect cochlear de-afferentation.

Acknowledgements

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